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THE OCCURRENCE OF A DIABETOGENIC FACTOR IN THE EYESTALKS OF CRUSTACEANS*

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During an investigation of an endocrine influence on carbohydrate metabolism of crustaceans, it was found that aqueous extracts of the eyestalks produced within a very short time an intense hyperglycemia in the blue crab, *Callinectes sapidus*. This observation had been made several weeks previously by Mr. J. Armstrong [1] in the crayfish, and it now appears that a diabetogenic factor may exist in the eyestalks of the decapod crustaceans at least, and that it may be an agent in the normal regulation of sugar metabolism in this group of animals.

Of the common large decapods of the Woods Hole area the lobster has been found to be a very suitable form for such an investigation, but owing to legal difficulties in obtaining this species the blue crab was used. Blood was obtained by heart punctures made through a small hole drilled in the carapace, and subsequently analyzed by the method of Miller and Van Slyke.[2] The use of an anticoagulant was found unnecessary. The blood sugar values represent the total reducing substances, and for the present no distinction between fermentable and nonfermentable sugar was made. The amount of blood withdrawn for each analysis was 0.5 cc.

The blood sugar values of crabs freshly brought into the laboratory vary considerably. As an example, the individual values of seven crabs were 16, 112, 52, 18, 60, 27 and 55 mgs. per cent. This considerable variation is due to the handling of the animals, for it disappears when the crabs are segregated into separate containers and left undisturbed. After 5 days of isolation and starvation, the blood sugar values fell gradually, so that on the fifth day the individual readings of the five crabs showing the highest of the above values were 25, 24, 22, 24 and 21 mgs. per cent. Excess handling of the animals or other forms of excitement tends to induce hyperglycemia even after segregation and fasting. Consequently, blood samples were obtained as rapidly as possible and with a minimum of disturbance to the animals. When extracts were to be tested for glycemic effects, the animals

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[1] Personal communication.

[2] Miller, B. F. and D. D. Van Slyke, 1936. *Jour. Biol. Chem.*, 114: 583.

were always separated into individual containers and starved for at least 5 days previously.

1. The Effect of a Single Injection of Eyestalk Extract

Twenty-one crabs were prepared as previously described. The average blood sugar value for this group was 20 mgs. per cent. The animals were divided into four groups of five animals each, so chosen that the average value of each group was 20 ± 1–2 mgs. per cent. Into each animal, 0.2 cc. of a sea-water soluble fraction of the eyestalks of *Uca pugilator* was injected, this volume being equivalent to one Uca eyestalk. Blood samples were taken from the first group one hour following injection, from the second group 2 hours after injection of this group, from the third at 3 hours after injection, from the fourth 4 hours after injection, and

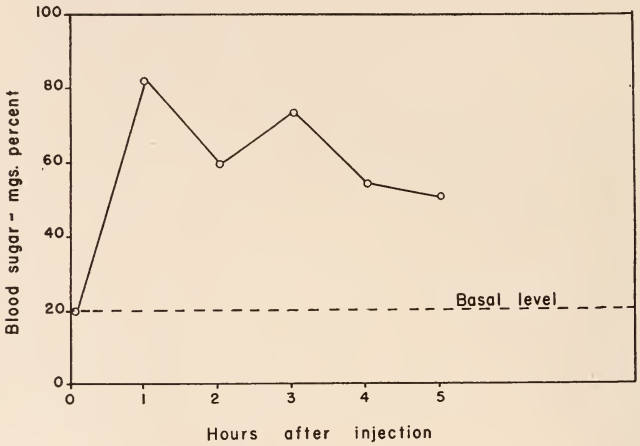

Figure 1. Curve showing the effect of a single injection of eyestalk extract (Uca) on the resting blood sugar level of the blue crab.

again from the first group 5 hours after the injection of this group. The results of this experiment are shown in Figure 1. The diabetogenic effect of the eyestalk extract is not due to reducing substances contained in the extract for, in the above experiment, the amount of extract injected into each animal contained only 1.3 mgs. per cent reducing substances calculated as glucose.

2. The Effect of Dilution on the Diabetogenic Action of Eyestalk Extracts

Eyestalks of *Uca pugilator* were ground thoroughly with a small amount of sea-water, the soluble portion decanted, and the residue re-extracted twice in the same fashion. The combined soluble portions were centrifuged, and from the super-

natant solution a series of dilutions ranging from 5 E.S.[3]/cc. of solution to 0.005 E.S./cc. solution was made. The amount of material injected into the test animals prepared as described above, was in all cases 0.2 cc. Blood samples were withdrawn from each specimen one hour following the injection. The results of this experiment are shown in Table I. It is evident that a rough agreement between

TABLE I

The effect of dilution on diabetogenic activity of the extracts

Number of animals injected	Dosage of extract	Blood sugar values—mgs. %
5	1.0 E.S.	82.4 ± 5.1
4	0.5 E.S.	59.5 ± 10.3
8	0.1 E.S.	49.5 ± 5.5
5	0.05 E.S.	47.0 ± 6.2
7	0.01 E.S.	46.4 ± 11.1
10	0.001 E.S.	41.6 ± 3.9
12	0.2 cc. sea water	24.5 ± 2.3
18	uninjected controls	20.0 ± 1.6

dosage and the resulting hyperglycemia exists, the greatest dose producing the greatest increment. However, there is little difference in the values obtained among the doses ranging from 0.1 E.S. to 0.001 E.S. The greatest response produced was a four-fold increase (1.0 E.S.) but it should be remarked that five- and six-fold increments in blood sugar values have been obtained with extracts of the eyestalks of Callinectes in the same, or even smaller doses. Injection of sea-water was without effect, the slightly higher value (24.5 mgs. per cent) for this group over the uninjected controls (20.0 mgs. per cent) being statistically insignificant. The extract was active in the lowest dosage given (0.001 E.S.) since the value obtained (41.6 mgs. per cent) represents a significant difference over either the uninjected or the sea-water injected control groups. However, some of the readings obtained with higher doses are not significant, this being due to the large standard deviation.

3. The Effect of Boiling on the Diabetogenic Effects of the Extract

Concentrated aqueous extracts of Uca eyestalks were divided into halves, one of which was placed in a water bath at 100° C. for several minutes, and the other left untreated. After heat coagulation, the boiled extract was centrifuged and the supernatant fluid diluted to the original volume before boiling. Both boiled and unboiled extracts were greatly diluted with sea-water so that each animal was injected with 0.2 cc. of a dilution containing 0.5 E.S./cc., or 0.1 E.S. total dose per animal. Blood samples were taken as usual one hour following injection. No difference was found in the activity of the boiled and unboiled extracts (Table II).

4. Localization of the Diabetogenic Activity of the Eyestalk

Four eyestalks of the blue crab were opened by a longitudinal incision, and the sinus gland of Hanström dissected out.[4] The glands were macerated in 4 cc. of

[3] The letters E.S. are used as an abbreviation of eyestalk.

[4] The position of the sinus gland was kindly demonstrated by Dr. F. A. Brown, Jr.

TABLE II

The heat stability of the diabetogenic factor

Dosage	Number of animals injected	Average blood sugar values—mgs. per cent	
		Boiled	Unboiled
0.1 E.S.	4, 4	56	53
0.1 E.S.	6, 6	61	54

sea-water and centrifuged. 0.25 cc. of the extract was injected into each of four animals, the amount being equivalent to one-fourth of a sinus gland. The eyestalks from which the sinus glands were removed were also macerated with 4 cc. of sea-water, centrifuged, and 0.25 cc. of the extract injected into each of four test specimens. One hour after injection, the average blood sugar of the group receiving the sinus gland extract was 83 mgs. per cent, and that receiving the eyestalk (minus sinus gland) extract was 30 mgs. per cent.

5. Effects of Extracts of other Tissues

Saline extracts of the hepato-pancreas of the blue crab were without effect on the resting blood sugar level. Similar extracts injected into specimens made hyperglycemic (100–120 mgs. per cent) by various means produced variable results; in two cases, a sharp fall was obtained while in the majority of cases insignificant changes were observed.

6. The Effect of Eyestalk Extirpation

Twenty-four crabs were isolated and starved for 3 days, after which time the eyestalks were surgically removed from 12 animals, the other 12 remaining as controls. The animals were then starved for another 7 days during which three sets of analyses were obtained from both normal and operated groups, on the second, fifth and seventh days following operation. The results are shown in Table III.

TABLE III

Animals	Blood sugar—mgs. per cent		
Days after operation	2	5	7
Normal (12)	22.6 ± 1.5	25.2 ± 4.5	22 ± 1.6
Operated (12)	24.4 ± 1.6	36.7 ± 4.6	40.3 ± 10.2

The blood sugar of the operated specimens tends to increase following the removal of the diabetogenic factor of the eyestalks, but the differences between the two sets of animals are not significant due to the large standard deviation. The results, however, seem paradoxical to those obtained from injection experiments, but it must be remembered that the operative injury may be sufficient to produce these high and

irregular values and thus to mask any possible hypoglycemia resulting from the removal of the eyestalk factor. This experiment should therefore be repeated with animals from which the sinus gland only is removed.

Summary

A powerful diabetogenic factor has been found in aqueous extracts of the eyestalks of crustaceans. The activity of the extracts is interspecific, heat stable, and effective over a wide dilution range. The sinus gland of Hanström appears to contain most of the diabetogenic factor.